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Sensitivity of Health Endpoints: Effect on Conclusions of Studies

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The types of biological response to an environmental agent can depend on dose, thus in this case a family of widely different dose-response relationships would be expected. For those situations where the magnitude of the effect may be determined on probabilistic grounds as a function of dose ("stochastic" model), no particular dose-response relationship may necessarily be inferred; the appropriate model still depends on the biological processes under consideration. Some examples of different conclusions concerning dose-response are given for studies of effects of lead and carbon monoxide at low doses. With increasingly sensitive measures of physiologic responses, these can be detected at exposures close to background, but for many cases the question remains whether an observed response really represents a true toxic effect. The application of epidemiologic data for regulatory purposes may depend on identification of the response to an agent appropriate for preventive measures. The conclusions one reaches about studies of health effects of environmental agents can be markedly influenced by the types of health endpoints under consideration.

I would like to begin by pointing out that agents that affect people can produce a variety of effects in them, and there may be very different sensitivities to these effects. That is, dose-response curves will vary greatly depending on which kind of response is observed, and traditional concepts of a "threshold dose" have stemmed basically from this fact. If you are exposed, let us say, to arsenic, it is rapidly lethal if you take enough of it. At lower doses it can produce a debilitating chronic disease which may cause the exposed person to die prematurely. Or arsenic may produce skin or lung cancer. The evidence suggests that skin cancer occurs at relatively high doses, with arsenic perhaps acting as a cocarcinogen in this case. We have new evidence in man that arsenic produces lung cancer at substantially lower doses. Thus, for many of the toxic agents that have been discussed at this symposium, a variety of dose-response curves could be anticipated, depending on the nature of the biological endpoint that is under investigation.

This concept is the thrust of what I want to talk about today. In the first case, the nature of the effect will be determined by the dose, as exemplified by arsenic. On the other hand, we can also have a situation where the biological effect is independent of the dose; that is, with bigger doses the probability of the effect occurring is increased, but the effect itself is unchanged. This is the type of dose-response relationship more commonly considered. A term used to designate this latter type of dose-response relationship is stochastic. The implication is that the events produced by exposure to an environmental agent are applied randomly within tissues or organs, and increasing the dose merely increases the probability that the event will occur. For example, if one looks at the binding characteristics of inorganic mercuric ion to cell surface enzymes, if the effect is determined by binding of mercury at a particular cell site, the probability of such binding could be governed by random statistics. On that basis, one might postulate that it would be a matter of how many surface enzymes were inactivated by the binding of mercury in order to produce the effect. This does not mean that because a random phenomenon is involved that the dose-response curve is necessarily a straight-line relationship, nor that there is no threshold.

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A number of other factors can influence the way in which environmental agents can produce various biological endpoints. For example, there are interaction phenomena with other agents in the environment. A good illustration is induction of the mixed oxidase enzyme, cytochrome P-450, in the liver by the ingestion of drugs; this induction may then modify the response of an individual to agents that could be more readily detoxified or modified by that enzyme system and thus reduce or increase the sensitivity to a potentially toxic material. Similarly, ingestion of ethyl alcohol can interfere with a number of liver reactions, which in turn can raise sensitivity to an environmental agent such as carbon tetrachloride. In this way, the shape of the dose-response curve may be modified, especially for low doses of the agent under study.

Finally, there are a number of host factors that can modify the response to an agent, such as age or genetic characteristics. For example, in relation to cancer induction, subsets of the population that have evidence of DNA repair deficiencies may be at particular risk, especially at low doses. Another example is when interference with oxidative metabolism is the effect of concern, the presence of anemia can modify the dose-response curve.

Thus, we should not look upon dose-response curves as immutably defined biologically for any particular environmental agent. The types of the dose-response curves will depend on the biological endpoints. A true threshold might be observed for responses dependent on interference with the activity of an enzyme. In many instances, we may have an excess of a particular enzyme required to handle reactions necessary to maintain cell function and, in this case, a toxic effect may require that the enzyme inactivation will depend on saturation for the effect to be observed. Or, if the agent affects the ability of the cells to replicate, for example, in the intestinal tract or the bone marrow, with their very rapid cell renewal systems, a kind of threshold may occur because the stock of stem cells may have to be depleted to a very low level before the ability of the stem cells to maintain tissue integrity is interfered with.

We also have to think in terms of the relationship of a cellular endpoint to the disease process that we perceive in man. It is all very well to talk about inhibition of an enzyme, but does such inhibition really mean that a toxic effect has been produced? I would like to give some examples where we have taken a critical look at this issue. For cancer induction, we often consider that the dose-response is a nonthreshold relationship; i.e., even a very small dose has an effect. The model is that following random statistics, carcinogens induce a change in the cell DNA which reads true, and therefore daughter cells continue to replicate in a damaged form which renders them more likely later of turning into a cancer. Even if that is the case, there may be mechanisms which make it possible either for these damaged cells to be repaired or, if not repaired, held in check or even eliminated, for example, by immune mechanisms. Thus the dose-response can be altered by these procedures.

I would like to illustrate some of the complexities of looking at specific biological endpoints, beginning first with lead, about which we know quite a bit.

Figure 1 shows the red-cell δ-aminolevulinic acid dehydrase (ALAD) enzyme activity studied in relation to the lead concentration in blood as a measure of the body burden. This is a semilogarithmic plot; thus the decline in activity is large. The point to note is that even at relatively normal blood lead levels, that is 20-30 μg/100 ml, there is a partial inhibition of this enzyme, which is related to the ability to manufacture hemoglobin in the bone marrow. It is one of the enzymes necessary for the production of the heme group in hemoglobin and lead blocks the action of the enzyme. So here is a case in which individuals with "normal" levels of lead show an effect. But is this inhibition a toxic effect? If so, the dose-response relationship from lead exposure would clearly have no threshold.

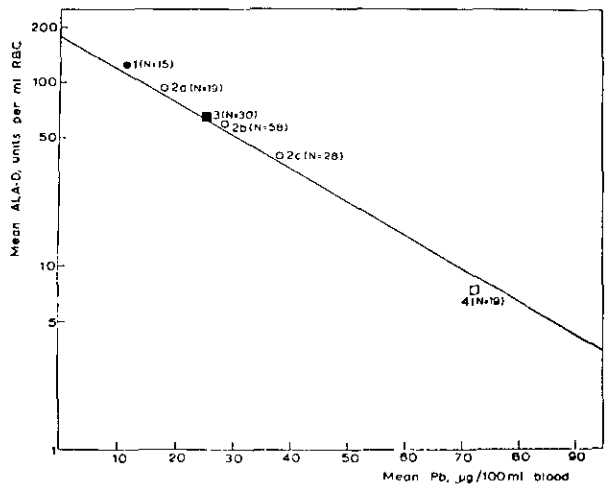

FIGURE 1. Relationship between δ-aminolevulinic acid dehydrase (ALAD) and blood lead: (●) healthy medical students; (○) printing shop workers, (2a) book binders, (2b) typesetters, (2c) stereotype metal setters; (■) automobile repair workers; (□) lead smelters and shipscrappers. Points are means for various groups, with the numbers in each group shown. The ordinate is a logarithmic scale. From Hernberg et al. (*1*) by permission.

Figure 2 shows a similar kind of study carried out on children in Belgium who went to school near a lead smelter. Here again blood lead is plotted at the same units, micrograms per 100 ml of blood. On the left-hand graph are the results in children for two responses. Plotted on the ordinate is the percent of children with elevated values of free erythrocyte protoporphyrin (FEP), and reduction in ALAD activity. The right-hand graph shows the results for FEP for the children and adult men and women. If these changes in prophyrin metabolism or enzyme activity truly mean that toxicity is present—and that is a controversial conclusion—then it is reasonable to conclude that the dose-response relationship is close to a straight line even at low levels of body burden.

From results such as these, we are now concerned with a range of effects of lead well below values that have generally been considered to be a toxic effect in the past. It may mean that the amount of lead that we have in our bodies could impair our ability to deal with, for example, hemorrhage or other conditions that might call upon increased red blood cell production. Thus in these terms, even background exposures are possibly producing toxic effects.

In the same way, from experiments carried out by Bingham some years ago, the number of free macrophages washed from the lung was found to

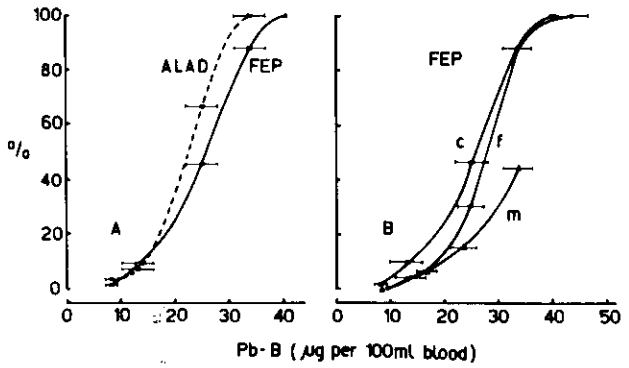

FIGURE 2. Relationship of the percent of individuals with abnormal indices to blood lead, showing ALAD (% of group with δ-aminolevulinic dehydrase activity less than 37 nanomole ALA transformed/100 ml erythrocytes) and FEP (% of group with free erythrocyte protoporphyrin greater than 83 µg/100 ml erythrocytes). These cutoff levels were determined from the 95% confidence limits for a control unexposed population. The abscissa shows the lead in µg/100 ml blood, with each point the mean of the blood lead by 10 µg/100 ml intervals, and the error bars representing the standard deviation of blood lead within that interval. Left (graph A) data for 143 exposed and unexposed children; right (graph B) % abnormal FEP for children (c) compared to those for adult women (f) and adult men (m). From Roels et al. (2) by permission.

be reduced if the animals were exposed to lead sesquioxide (3). The effect could be detected at exposures of 10 $\mu g/m^3$, which is not much above the levels that are present in some urban areas.

In subsequent experiments, macrophages were washed from the lungs of exposed animals and they were then incubated for a period of time. If the control cells were incubated with the wash-out fluid from the rats exposed to lead sesquioxide, the control cells were lysed, indicating something was produced in the wash-out fluid which led to a cytotoxic phenomenon. These observations indicated that the reduction in free macrophages could be accounted for by this lytic effect of lead exposure.

The significant point of these findings was that in the whole animal this effect was dose-independent. That is, a Pb_2O_3 level of 10 $\mu g/m^3$ was about as effective as 150 $\mu g/m^3$. Thus any dose dependence was at extremely low concentrations. These results again indicate quite complex dose-response relationships and show that very low doses could be important if these lytic effects on macrophages are significant in terms of defenses against inhaled microorganisms.

I would now like to leave lead and close my discussion by considering carbon monoxide, another very ubiquitous agent, and one which EPA is concerned about as well. Figure 3 is from a study done by Ayres et al. (4), in which a small dose of carbon monoxide was brought rapidly into the arterial system of human patients and a number of cardiac metabolic factors were measured. Note the results obtained in the patient with coronary artery disease: with a rise of about 6% carboxyhemoglobin (COHb), coronary sinus oxygen showed a drop of about 40%, and coronary blood flow showed a slight increase because this individual had coronary artery disease. Most significant, however, was the clear indication that the myocardium was switching from oxidative to anerobic metabolism, as judged by the fact that both pyruvate and lactate, instead of being extracted from arterial blood after COHb was elevated this small degree, were excreted into the venous blood. The patients with mitral stenosis and emphysema showed similar responses to somewhat higher levels of COHb. The observations by Aronow et al. (5) in patients with angina pectoris, are consistent with these metabolic changes from small concentrations of COHb.

Figure 4 shows the decrease in maximum oxygen uptake in healthy subjects exercised on a treadmill after exposure to carbon monoxide. Under the influence of elevated carboxyhemoglobin, a significant reduction in the maximum oxygen up-

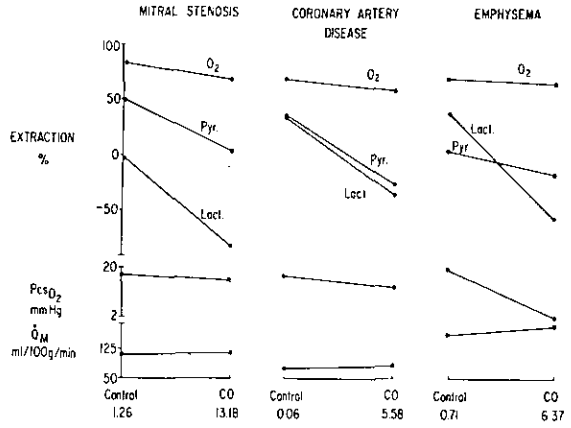

FIGURE 3. Effects of a small sudden increase in carboxyhemoglobin, induced by brief exposure to 5% carbon monoxide in air, observed in patients with cardiopulmonary disease. Extraction % denotes percent myocardial extraction of oxygen (O_2), pyruvate (pyr.) and lactate (lact.). Note that a negative "extraction" means that the coronary sinus lactate or pyruvate was higher than in arterial blood. Pcs_{O_2} denotes coronary sinus oxygen tension (in mm Hg); $\dot{Q}m$ denotes coronary blood flow (in ml/100 g/min). The abscissa shows blood carboxyhemoglobin % for control and test conditions. Carbon monoxide decreased oxygen extraction, and the outflux of lactate and pyruvate indicated anaerobic metabolism by the myocardium. From Ayres et al. (*4*) by permission.

take occurred because of the interference of oxygen transport by COHb. The decrease in oxygen delivery extends down to values of about 3% COHb. There is little indication of a threshold to this particular effect. But, again, is this a significant physiological change? That is, is a few percent change in the maximum oxygen uptake a significant toxic effect? I leave that to you to decide.

Figure 5 shows in the upper graph the concentration of carbon monoxide in the air of Los Angeles during three days in January. In the bottom graph are the levels of CO, calculated from the well-known equations governing uptake of carbon monoxide, plotted for the arterial blood of a mother and of her fetus in utero. Although the fetal blood tends to lag behind the maternal blood values, both are about the same. The results indicate about 4% carboxyhemoglobin would be present in the fetal blood.

Figure 6 gives the result of animal experiments carried out by Longo et al. (*8*) in which the oxygen pressure in the fetal descending aorta and in the inferior vena cava of the fetus was measured and expressed as a function of the carboxyhemoglobin in the fetal blood. Oxygen partial pressure is progressively reduced as COHb increases. If a reduction of tissue oxygen pressure is potentially

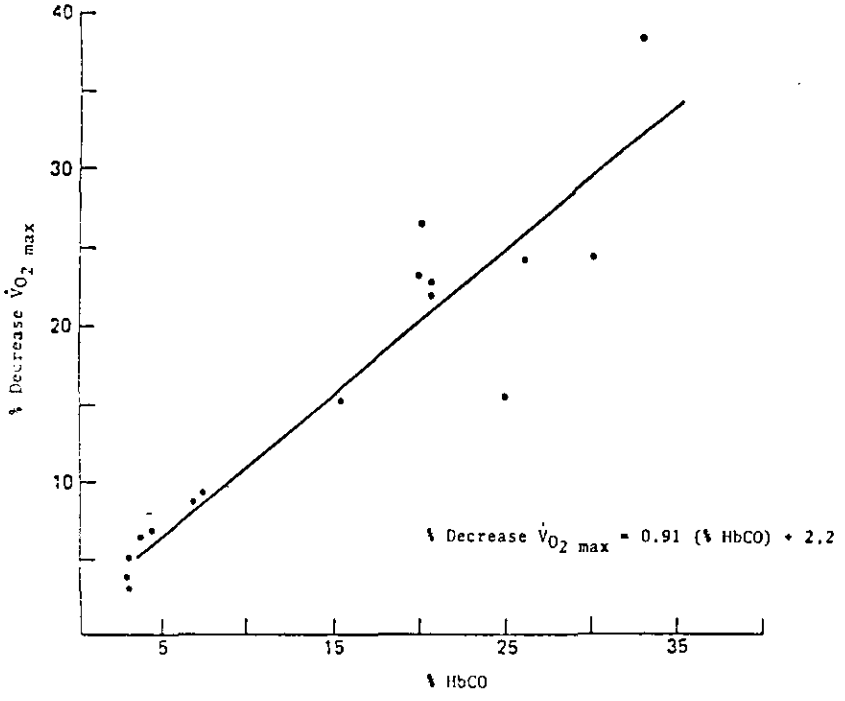

FIGURE 4. Percent decrease in maximum oxygen consumption ($\dot{V}_{O_2\ max}$) vs. percent carboxyhemoglobin in healthy subjects. The regression line and equation are also shown. Data of S. M. Horvath (*6*) by permission.

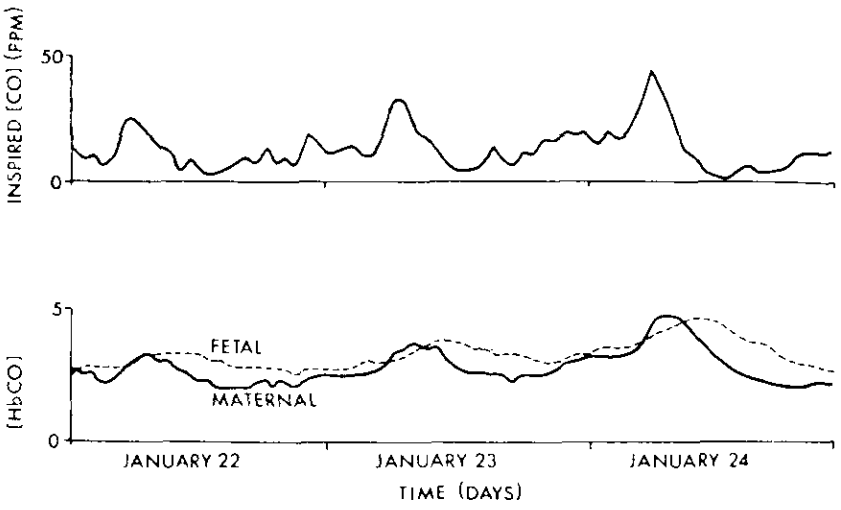

FIGURE 5. Plots of (top) inspired carbon monoxide concentration (in ppm) from measurements of ambient air during a 3-day period in southern Los Angeles and (bottom) calculated maternal and fetal arterial carboxyhemoglobin concentration [HbCO] for a pregnant woman exposed to these values of ambient CO. After each peak in inspired CO, the fetal [HbCO] remains elevated for long periods of time. From Hill et al. (*7*) by permission.

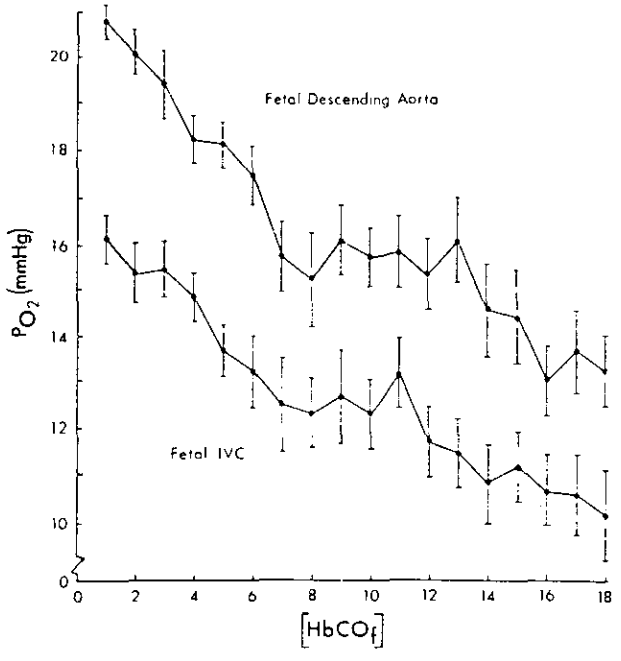

FIGURE 6. Oxygen pressure P_{O_2} (in mm Hg) in descending aorta and inferior vena cava (IVC) of sheep fetuses, as a function of elevations of fetal carboxyhemoglobin induced by exposing ewes to carbon monoxide. From these values of arterial and venous P_{O_2}, a reasonable conclusion is that tissue P_{O_2} is inversely related to fetal carboxyhemoglobin, with no threshold. When fetal carboxyhemoglobin reached more than 15% for 30 min, over half of the fetuses died. From Longo (*8*) by permission.

significant in interfering with fetal development, these results suggest that the dose-response relationship for CO exposure could be expressed by a linear no-threshold dose-response curve. Even at very low values of COHb the tissue oxygen and hence optimum development of the fetus may be compromised.

Studies have been carried out by Horvath et al. (*9*) in which they required adult subjects to respond to a complex set of signals. The task involved being alert to a number of cues before a response was made; that is, the test was of continuing vigilance. Figure 7 gives the percent of correct signal responses against time in minutes, for three levels of CO exposure. For 26 ppm CO, or at 2.6% COHb, little effect was observed, but at 111 ppm or at 6.6% COHb, a reduction of correct responses occurred. These results suggest that there is a quasi-threshold for this type of central nervous system effect of CO.

I have tried to illustrate from a variety of situations that we do not necessarily expect to find the same dose-response curve applying to all biological endpoints. My emphasis has been on physiological endpoints of toxic responses, including effects on the central nervous system. In closing, I think it is appropriate to mention that there is a kind of dose-response curve about which we have virtually no data, and that has to do with the perception of people about their environment. I took part many years ago in a symposium on annoyance reactions to environmental effects, such

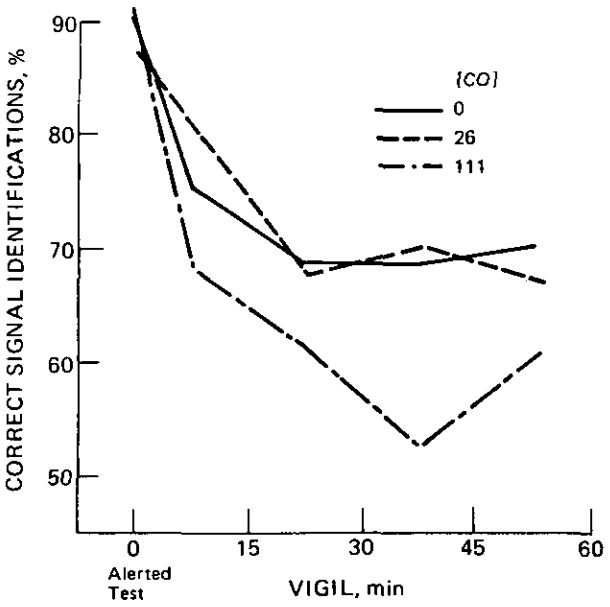

FIGURE 7. Vigilance performance (% correct signal identification) vs. time test carried out, for three levels of inspired carbon monoxide given for one hour prior to and during the test. Subjects were 10 nonsmoking young male volunteers, each of whom was exposed randomly and without knowledge of experimental treatment to all three levels of exposure. Final carboxyhemoglobin values averaged 0.8%, 2.3% and 6.6% for the three levels of exposure (0, 26 and 111 ppm CO). From Horvath et al. (*9*) by permission.

as from odors, noice or pollution in general. How much of this can be quantitated in human populations? We know that people who live around kraft paper mills, for example, object to the characteristic paper mill odor. Can we get quantitative estimates of dose-response relationships for responses to environmental insults of this type? I think one of the things emphasized by several papers in this symposium is that perceptions of people may be just as important in terms of potential health effects as pure toxicologic or biological endpoints that we are so fond of trying to quantitate in studies such as those cited. This field of psychological impacts of environmental exposures has scarcely been touched up to the present.

In conclusion, I think we must recognize that dose-response relationships are approximations, and that one of the responsibilities of the epidemiologist and the experimentalist is to try to define as well as possible a biologic basis for determining what the appropriate model is likely to be. It is clear that the conclusions one reaches about studies of health effects of environmental agents can be markedly influenced by the types of health endpoints under consideration.

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